CARBON MONOXIDE A RESPIRATION PRODUCT OF NEREOCYSTIS LUETKEANA

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(WITH THREE FIGURES)

The consideration of carbon monoxide as a plant respiration product is somewhat novel from the point of view of the plant physiologist. That such may be the case seems clear from the evidence to be submitted in this paper. This investigation was carried on during the summer of 1918 at the Puget Sound Marine Station at Friday Harbor, Washington. It was expected to continue the investigation for another summer before publication. This was not possible, however, and since it now appears wholly unlikely that the work will ever be carried further by the authors, the data at hand are presented.

In previous papers by LANGDON,[1] it was shown that there was present an average of 4 per cent (by volume) of carbon monoxide in the pneumatocyst of the giant Pacific Coast kelp, *Nereocystis Luetkeana*. This statement was based on the analysis of the gas from about 1000 different specimens. The quantity of carbon monoxide varied from 1 to 12 per cent, the average being 4 per cent. The actual existence of the carbon monoxide in the gas from the kelp was demonstrated by a variety of chemical tests, by its physiological effects on animals, and by the standard spectroscopic blood tests. There was also present in the gas 15–25 per cent of oxygen, the remainder of the gas being nitrogen. There was no evidence of the presence of other gases, except, of course, water vapor, although particular search was made for carbon dioxide, hydrogen, and hydrocarbon gases.

The occurrence of carbon monoxide within a living plant at once raised the question as to its source and possible relation to anabolic or katabolic processes. In the theoretical[2] considerations

[1] Jour. Amer. Chem. Soc. **39**:149. 1917; also Puget Sound Marine Sta. Publ. **1**:237. 1917.

[2] SPOEHR, H. A., Plant World **19**:1. 1916.

of the mechanisms of photosynthesis, carbon monoxide has often been considered as an intermediate step in the reduction of carbon dioxide, especially since it is so closely related chemically to formaldehyde and formic acid. Heretofore there has never been any evidence of the existence of free carbon monoxide within a living plant. The possibility of its formation by enzyme action or by decay processes was early suggested, and was the first point investigated. Finely ground kelp was allowed to undergo autolysis in contact with sea water, and the gases evolved were examined. No carbon monoxide was formed, but the gas consisted almost entirely of carbon dioxide and hydrogen.

The next step was to determine how rapidly carbon monoxide was formed within the living plant. The method of work and the further discussion will be made more clear if prefaced by a brief description of *Nereocystis*. Fig. 1 shows the kelp as it lies normally, almost submerged in the sea water, anchored to the rock bottom and supporting the streaming fronds from the top of the hollow gas-filled stipe. The plants vary greatly in size. Individuals are often 80–100 ft. in length, and contain several liters of gas which is usually at reduced pressures.[3] The inside of the gas cavity is relatively dry, and is lined with a delicate weblike structure called sieve tubes. The plant will withstand a great deal of mutilation and still continue to live and grow, if kept in sea water.[4]

It was found practicable to cut off the lower part of the stipe and in the upper part to substitute a gas of known composition for that normally present in the pneumatocyst. The cut end was closed by a cork stopper, and the plant weighted and submerged in the sea, tied to a floating support, as shown in fig. 2. After a suitable interval, changes in the gas composition were determined by analysis. In the first experiments tried, primarily to determine the rate of formation of carbon monoxide, air was substituted for the kelp gas. This was accomplished by filling the cut stipe with sea water and then emptying. This process repeated three or four times removed the small bubbles that tended to adhere to the delicate sieve tube lining of the pneumatocyst, and insured the

[3] FRYE, T. C., Puget Sound Marine Sta. Publ. 1:85. 1916.

[4] FALLIS, A., Puget Sound Marine Sta. Publ. 1:1. 1916.

complete removal of all the gas originally present. The cut end of the now air-filled stipe was corked and anchored at the surface of the sea as previously described.

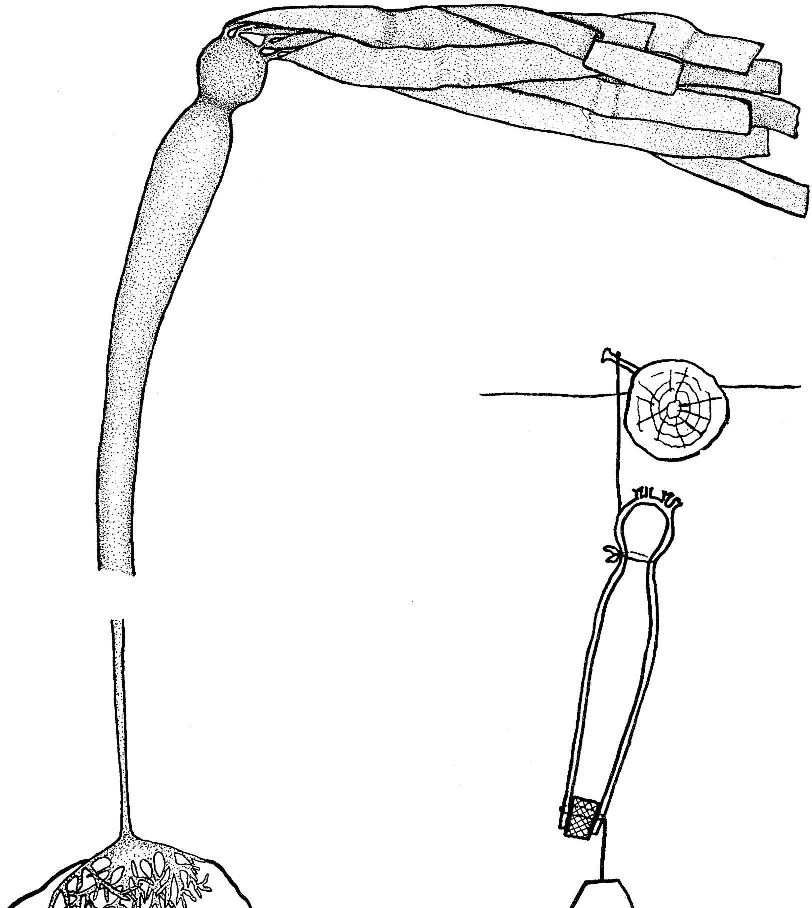

FIG. 1 FIG. 2

Analysis of the gases from a series of these cut and air-filled plants was made after various intervals of submergence. The typical data given in table I show clearly the gradual formation of carbon monoxide, accompanied by the appearance of carbon dioxide, which latter was undoubtedly due to decay processes,

since carbon dioxide is not found in the gas normally present within the kelp. In general, the cut and corked sections of stipe remained sound enough to be tight for a week or ten days, although evidence of local decomposition was apparent. This production of carbon monoxide, when the stipe was filled with air, was confirmed by many determinations with different specimens. In most cases it appeared in quantities as great as 1 per cent or more. The presence or absence of the fronds had no relation to the carbon monoxide formation. Carbon monoxide was produced by sections cut from any part of the hollow stipe, filled with air, corked, and similarly suspended in the sea. Even the round bulblike top of the stipe,

TABLE I

Time	Percentage CO_2	Percentage CO	Percentage O_2
Start	0.0	0.0	20.8
24 hours	0.3	0.0	16.5
48 hours	0.0	0.4	13.0
73 hours	0.6	1.0	7.0
97 hours	1.0	3.2	6.2
110 hours	1.1	4.5	5.0

devoid of fronds, would form it almost as readily as if practically the whole of the plant were used.

Two other methods of displacing by air the gas originally contained within the kelp were used, but gave no difference in the final results. The first of these was to insert a rubber tube at the cut end, so that it extended the whole length of the stipe and up into the bulblike top; then to force in through the tube a large quantity of air, and thus sweep out all of the kelp gas. The other method was to draw the original gas out by connecting the cut end to a good suction filter pump. Alternately evacuating and filling with air served to accomplish the desired substitution without getting the inside of the gas cavity wet. It would be interesting to make the substitution under strictly aseptic conditions, but this the authors were not able to do.

Since carbon monoxide was formed in quantity in the living plant within a few days and was not formed by decay or autolysis, it might have been formed either as a product of respiration or as

an intermediate step in photosynthesis. If the latter, it should not appear if the experiments were carried out in the dark. To test this, boxes were constructed which were light-tight, but which would allow a ready flow of water through them. These boxes were 1 ft. square and 18 ft. long. The ends were closed by light traps, the baffle boards of which were inclined in the direction of flow of the water (fig. 3). The lids were also light-tight. All

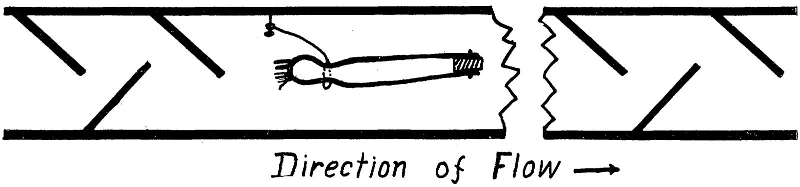

FIG. 3

holes and cracks were closed with pitch, and the whole interior painted a dead black. The boxes were weighted so as to just float; the waves washed entirely over them except when the water was perfectly calm. The boxes were anchored in the bay (Friday Harbor, Washington) where the tidal currents are heavy, so that there was practically always a flow of water through the boxes. The boxes were large enough to hold several specimens without impeding the flow of water.

In the first experiments, the top 18 inches of stipe from 10 kelps were filled with air by displacement with sea water and corked and placed in the dark boxes. Half of them had the fronds removed. After 5 days in the dark they were removed and the gas analyzed. All showed carbon monoxide. The range was from 0.4 to 1.7 per cent, with an average of 0.7 per cent. The 20.9 per cent of oxygen in the air with which they had been filled had practically disappeared, and there was about 4 per cent of carbon dioxide. The oxygen was used by respiration and decay processes. These data were checked by extended experiments, and it was made certain that in the dark as well as in the light carbon monoxide was formed regularly in the air-filled section of the stipe, and that there was no relation between its appearance and the presence or absence of the fronds.

The appearance of CO_2 and the lowering of the oxygen content when the unmutilated plants were kept in the dark for some time were shown by the following experiments. Twelve whole plants were gathered from the same bed, precaution being taken to avoid in any way disrupting the gas cavity. The gas from 6 of them was analyzed at once and showed an average of 15 per cent of oxygen, 3.2 per cent of carbon monoxide, and no carbon dioxide. The other 6 plants were placed intact in the dark boxes, and after being anchored out in the tidal currents for 6 days showed the following average gas composition: oxygen 4.7 per cent, carbon monoxide 2.9 per cent, and carbon dioxide 0.5 per cent. There was therefore a marked decrease in the oxygen content, and an appearance of carbon dioxide which is practically never present in the kelp when freshly collected, while there was practically the same carbon monoxide content.

The substitution of gases other than air for those normally present was next undertaken. As a result of more than 40 carefully made experiments, in which nitrogen was substituted for the kelp gas, it can be stated confidently that no carbon monoxide was formed, either in the light or in the dark, either when fronds were present or when they had been removed, or at any intermediate time between the initial filling and the 8–10 days before decay had become so pronounced that observations could no longer be made. It should be remarked that a small percentage of carbon dioxide was generally formed, even though there was no oxygen present.

The nitrogen was prepared in three ways. One method was by heating a mixture of concentrated aqueous solution of sodium nitrite and ammonium chloride. The products other than nitrogen from this reaction are sodium chloride and water. The first few liters of gas evolved were discarded and the remainder showed no oxygen. The gas was washed through strong sodium hydroxide solution. The second method of preparing "nitrogen" was to absorb the oxygen from the air by means of alkaline pyrogallol. That the failure of the plant to produce carbon monoxide was not due to a trace of some unknown impurity, introduced into the nitrogen as chemically prepared and purified, was made certain by the use of nitrogen obtained by the fractional distillation of liquid

air. This nitrogen procured from the Linde Air Products Company contained a trace of oxygen, about 6–8 parts per thousand by volume, but the results obtained with it were the same as with the nitrogen prepared in other ways.

Similar experiments were carried out in which hydrogen was substituted for the kelp gas. The 15 determinations made showed no formation of carbon monoxide within 5–7 days, either in the light or in the dark. Here, as in the case of the nitrogen-filled kelp, a small percentage (1–9 per cent) of carbon dioxide was formed. It should be remarked that there was always a marked reduction in pressure for hydrogen-filled kelp. This amounts to an absorption or diffusion out of hydrogen. The whole relation of hydrogen in this connection deserves more exhaustive study.

The hydrogen used was from two sources: (1) the action of dilute sulphuric acid on the so-called arsenic free zinc; (2) a commercial product prepared by electrolysis.

A number of sections of kelp stipe were filled with a mixture of nitrogen and oxygen, both chemically prepared. The initial composition was 15.2 per cent oxygen and the remainder nitrogen. After 6 days' exposure carbon monoxide had been formed in all cases, the quantities ranging from 0.8 to 2.1 per cent. The oxygen content decreased and some carbon dioxide was formed just as in the case of the specimens filled with air. Kelp filled with a mixture containing 26.2 per cent of oxygen and the rest hydrogen showed in the same time a similar formation of carbon monoxide and a corresponding decrease in oxygen accompanied by the appearance of carbon dioxide.

The evidence so far presented seems to point to the inevitable conclusion that the carbon monoxide is a product of respiration, since it is not formed by decay or autolysis, and is formed only when there is oxygen present in the pneumatocyst.[5]

A similar supplementary series of experiments was made which supported the conclusion that the carbon monoxide was formed by a katabolic process. The gas-filled corked sections of

[5] That the CO was a respiration product was early suggested by RIGG. This conclusion was based on a special knowledge of the physiology of the plant. It was his belief that the effect was intimately related to the sieve tubes.

stipe were not put back in their normal habitat, sea water, but were left out in the air. It should be recalled that this plant will withstand a very considerable amount of desiccation and will resume growth when returned to the sea. Sections of stipe filled with air and placed in a warm, dry, dimly lighted attic developed 0.7–4.7 per cent of carbon monoxide in 5 days. The oxygen content lowered to about 4 per cent and there appeared 1–2 per cent of carbon dioxide. Similar results were obtained when specimens of air-filled kelp were placed in good daylight (not direct sunlight) or when kept exposed to the air in a dark room. Kelp filled with nitrogen or with hydrogen and allowed to stand (dry) in the air developed no carbon monoxide. On the other hand, under the same conditions, but filled with mixtures of oxygen and hydrogen, or oxygen and nitrogen, carbon monoxide was produced within a few days.

That carbon monoxide was not formed in dead kelp was shown in the following manner. A number of plants were killed by being placed for 10 minutes in sea water which was maintained at a temperature of 50° C. The stipes, full of air, were corked. Some of them were placed in the sea water in the light and some anchored out in the dark boxes. Another set similarly treated was placed in air, some of them in the light and the remainder in the dark. Analysis of the gas from the various specimens 6 days later showed no carbon monoxide. There had been a slight decrease in the oxygen content and the formation of 2 or 3 per cent of carbon dioxide. Exactly similar results were obtained when the kelp was killed by being placed in N/50 $Cu\ SO_4$ for 18 hours.

A series of experiments was started to determine what would happen if kelp were filled with oxygen-free nitrogen or hydrogen to which had been added a small quantity of carbon monoxide. These experiments were not completed, but it became clear that the change, if any, was slight.

Nereocystis Luetkeana seems to be remarkably well adapted to research on gas exchange of living cells. By the use of the very refined methods of gas analysis which are now available, some very interesting and valuable information might be gained. It is possible that traces of hydrogen or carbon monoxide not revealed

by the technical gas analytical methods used in this work may be playing important rôles in plant processes.

The gas contained in the hollow cavities of several other growing plants was investigated, but in no case was carbon monoxide found. The gas obtained from a vigorously growing garden pumpkin contained 18 per cent of oxygen with no carbon dioxide or carbon monoxide. Very similar results were obtained from the gas contained in the hollow stems of a species of *Equisetum*, the hollow stems of a species of *Petasites* (colt's foot), from the pods of green garden peas, and from the seed pod of the soft maple. In the original paper it was reported that there was no carbon monoxide in the gas obtained from the vesicles of *Egregia Menziesii*, or in that obtained from *Fucus evanescens*.

The Scripps Institute collected gas for the author from two of the southern algae. One of these, *Macrocystis pyrifera*, showed no carbon monoxide; while the other, *Pelagophycus Porra*, showed a small carbon monoxide content. These gases were collected in quart fruit jars and shipped from La Jolla, California, to Seattle, Washington, for analysis. To be definitely certain of the presence of carbon monoxide in the gas from the elk kelp, *Pelagophycus Porra*, it would be necessary to examine the freshly collected gas. It is interesting to note that *Pelagophycus Porra* is very closely related to *Nereocystis Luetkeana*, and is very similar in structure, the inside of the gas cavity of both being characterized by the presence of a pith web.

The occurrence of free carbon monoxide within a living plant is unique, so far as the authors have been able to ascertain. Its further study in relation to plant life should prove interesting, and it is not without a measure of regret that the authors leave this field to other investigators.

Summary

1. The existence of a percentage of carbon monoxide in the gas contained in the pneumatocyst of the Pacific Coast kelp *Nereocystis Luetkeana* is confirmed.

2. The substance of the kelp when ground and allowed to undergo autolysis and decay does not form carbon monoxide by enzyme action or fermentation process.

3. Kelp plants, in which the gas normally present within the floater is replaced by air, form several per cent of carbon monoxide within a few days.

4. The formation of carbon monoxide takes place only when oxygen is present as one of the gases within the floater. No carbon monoxide is formed when the floater is filled with nitrogen or hydrogen.

5. Light does not affect the rate of formation of carbon monoxide.

6. The gas obtained from the cavities of various other plants failed to show a similar occurrence of free carbon monoxide.

7. The percentage of free carbon monoxide which occurs in the floater of *Nereocystis Luetkeana* is considered to be a respiration product for the following reasons. It forms only when oxygen is present within the floater; it forms as readily in the dark as in the light; it is not formed by enzyme or fermentation process when the substance of the plant undergoes autolysis and decay; and it is not formed in killed plants.

In conclusion, the authors wish to acknowledge the many courtesies extended to them by Dr. T. C. Frye, Director of the Puget Sound Marine Station.

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